actions. These requirements are set forth in the basic regulatory statute such as the Clean Air Act, the Toxic Substances Act, or the Atomic Energy Act; in the Administrative Procedure Act; and of great importance in recent years, the National Environmental Policy Act. In addition, however, the courts, particularly the U.S. Court of Appeals for the District of Columbia, have attempted to fashion procedural requirements of their own. For example, in a series of decisions that court has insisted upon "principled decision-making" by the regulatory agencies. This requires "administrative officers to articulate the standards and principles that govern their discretionary decisions in as much detail as possible."[23]

The judicial decisions that invalidate agency actions on procedural grounds may reflect an implicit disagreement with the substantive position reached by the agency. Indeed, Judge Wilkey of the D.C. Circuit openly accused his colleagues of using the National Environmental Policy Act "to delay the development of important energy sources."[24]

In terms of explicit review of the substance of the agency's action, the courts are required to sustain the agency's action if there is "Substantial evidence" supporting it. "Substantial evidence" does not mean the weight or preponderance of the evidence, but only that there was enough reasonable evidence to support the agency's findings. Thus, the court cannot invalidate the action merely because it disagrees with it or believes it was incorrect. It is not necessary that the evidence before the agency be certain. As the D.C. Circuit stated in one case in which it upheld a decision by the EPA Administrator in which there was a "great mass of often inconsistent evidence," the "evidence is substantial enough to support the conclusions of the administrator, although it possibly might support the contrary conclusions as well."[25]

I return to somewhere in the vicinity of where I began in my discussion of the legal culture. The judicial system relevant to environmental decision-making simply is not equipped to, and is not expected to, produce objectively correct decisions. It is, however, expected to guard against decisions that are arbitrary, capricious, or, indeed, "far out." A useful way to look at the process is as part of the political system. The decision-making power of regulatory agencies is constrained in three ways. The members of Congress who were instrumental in enacting the environmental legislation in the first place usually have a paternalistic interest in having the legislation implemented in the general manner they intended. A "far out" action by the agency is likely to produce some kind of a formal legislative action such as a corrective amendment to the statute or informal action such as strong public rebukes, hostile hearings, appropriation cuts, or the like.

A second constraint, also political in nature, is the fact that the agency is subject to the influence of the President in a number of respects. Even where the agency is an "independent regulatory agency" which Congress established with safeguards to insulate it from control by the President, he may nevertheless be able to exercise some control through the budgetary process and his/her power to nominate, and in some cases remove, the agency heads. The agency heads cannot, therefore, stray too far from the President's political desires lest they jeopardize prospects for their reappointment and advancement in the government service.

The third constraint is the likelihood of judicial action overturning the regulatory decision.

Reviewing courts are subject to similar constraints: the possibility of corrective legislation and of reversal by the Supreme Court. Although they are much less politically vulnerable than regulatory agencies, they too must reach their decisions with one eye focused on political reality.

Thus it is concluded that the courts' role in environmental decision-making is quite

limited and tends to center more upon procedural than substantive issues. Because the courts have no scientific competence, contestants in environmental decision-making who come before the courts are compelled to do so in the vocabulary of ordinary discourse; to try to reduce scientific information to language that can be comprehended by laymen (even though the courts and laymen may often in fact erroneously comprehend). It must be remembered that in environmental litigation, the basic issues involve benefits and risks to the public, the assignment and allocation of which is essentially a political function. Even though such issues are decided in the first instance in the legislative process, the role of the courts is essentially to assist in the resolution of disputes that arise over the application of statutes in particular situations. In this sense, therefore, the role of the courts is adjunctive to and supportive of the democraic political process. We cannot, therefore, expect that the environmental decisions of the courts will be regarded as scientifically acceptable, let alone scientifically correct.

Some will find this conclusion uncomfortably pessimistic. Although everyone will, at least on a moment's reflection, agree that the legislative process is essentially political, and that it frequently produces strange or seemingly irrational results, we seem to expect better results from our courts, and by and large I think we get them. But why should we expect a more objectively correct decision on a scientific issue from a court that is interpreting and applying an environmental statute that was enacted by Congress through the essence of the political process with little attempt to ensure that the statutory standards reflect good or objectively correct science?

References Cited

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2. 583 F. Supp. 247 (D. Utah, 1984).
3. These comments, and those quoted below, were made by Judge Bruce S. Jenkins, before whom the case was tried, in a talk at the Fourteenth Annual Conference on the Environment sponsored by the ABA's Standing Committee on Environmental Law on May 17–18, 1985. See American Bar Association Standing Committee on Environmental Law, *Dealing With Risk: The Courts, the Agencies, and Congress* 1 (1985).
4. *Ibid.*
5. *Allen v. United States*, *supra* at 257–58.
6. **Jenkins,** *op. cit. supra* n.3, at 2.
7. *Id.*, at 2–3.
8. *Ibid.*
9. *Ibid.*
10. *Id.*, at 4.
11. *Id.*, at 3.
12. Restatement of the Law of Torts, 2d, §291.
13. *Id.*, §293.
14. *Id.*, §292.
15. See, for example, *Osborn v. Montgomery*, 203 Wisc. 233, 234 N.W. 372 (1934); *United States v. Carroll Towing Co.*, 159 F.2d 169 (2nd Cir. 1947).
16. **Jenkins,** *op cit. supra* n.3., at 4.
17. 499 F. 2d 467, 474; see also *Ethyl Corp v. Costle*, 541 F.2d. 1, 26–29. (1976).
18. *Ibid.*
19. National Academy of Sciences, *How Safe is Safe? The Design of Policy on Drugs and Food Additives,* 1, 2–3 (1974). Dr. Handler's comments were made in his remarks opening the forum.
20. 5 U.S.C.A. §551, et seq.
21. See n. 1, supra, and accompanying text.
22. 21 U.S.C.A. §348(c)(3)(A).
23. *Environmental Defense Fund v. Ruckelshaus,* 439 F. 2d 584, 598 (1971).
24. Dissenting opinion in *People Against Nuclear Energy v. Nuclear Regulatory Commission,* 678 F. 2d 222, 237–238 (1982).
25. *Environmental Defense Fund v. EPA,* 489 F. 2d 1247, 1252 (1972).

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BOOK REVIEW

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The Encyclopedia of Aquatic Life. Edited by K. Banister and A. Campbell. Facts On File Publications, New York. XXXIII + 349 pp. Price $35.00.

My major criticism of this book is its title, which implies a broader coverage of aquatic organisms than is delivered. This is a book about only certain groups of freshwater and marine animals. In the preface, the editors explain that amphibians are not included because they only "return to the water to breed" and pinnipeds are omitted because they "leave the sea to breed on land." Plants and bacteria also are not covered. I will have more to say about this later.

The book is intended for a general audience. It is divided into three parts. In each part, the discussions are organized around broad taxonomic groupings. The first part covers freshwater and marine fishes. The second part surveys invertebrates, excluding insects and spiders but, for some reason, including many parasitic forms. The third part of the book describes whales, dolphins and sea cows. At the start of each chapter, information on the taxonomy, geographic distributions and size ranges of each group is summarized concisely in boxes. There is a short glossary and index at the end.

Twenty-eight scientists are listed as contributors to the book. The editors have done a good job of maintaining a consistent style throughout. The text is relatively free of errors and does not overgeneralize. On the whole, I thought the parts on fishes and marine mammals were more successful. I particularly enjoyed in the first part the anecdotes, such as Julius Caesar's interest in moray eels (p. 27) or how some cyprinds become drunk by gorging themselves on fermented fruit (p. 79).

A strong point of the book is its illustrations. Most of the photographs and drawings are in color. They complement well the text. I often found myself flipping through the pages simply to look at the pictures, many of which are stunning.

Without photosynthetic production by plants, none of the animals in this book could exist. Plants are an essential part of freshwater and marine systems, and I wonder why the editors did not include them in a book claiming to be an encyclopedia of aquatic life. There is only one short paragraph on marine phytoplankton in the section on zooplankton (p. 154), and seaweeds and aquatic angiosperms are only mentioned incidentally as food and habitats for various animals. They are not even given listings in the index. I also

find it inconceivable that seals are not discussed. A section on pinnipeds should accompany the chapters on other marine mammals.

Because this book does not live up to its title, I can give it only a qualified recommendation. Serious amateurs and students will find much useful information on the animal groups that are covered. The text is very readable and the illustrations outstanding.

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Increased Uptake of Thymidine in the Activation of Sea Urchin Eggs: IV. Effects of the Nucleoside Transport Inhibitor, Nitrobenzylthioinosine

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ABSTRACT

Uptake of thymidine in *Strongylocentrotus purpuratus* eggs increases greater than 50-fold shortly after fertilization. This uptake is inhibitable by nitrobenzylthioinosine (NBMPR). Binding of NBMPR to surface sites on fertilized eggs is not Na^+-dependent. Binding to parthenogenetically activated eggs in Ca^{2+}-free sea water (OCa^{2+}-SW), however, reveals a Ca^{2+}-dependent component. Measurements of thymidine uptake in OCa^{2+}-SW also reveal a Ca^{2+}-dependent component. Along with its inhibitory effects on thymidine uptake, NBMPR exerts inhibitory effects on cleavage and early embryonic development.

Introduction

It has long been thought that the overall activation of the sea urchin egg into development starts with structural and functional changes at the cell surface. This idea follows naturally from observing the first events of fertilization and led the earliest investigators to advance the "permeability theory"[1] or to describe fertilization as a "cytolysis of the cell surface."[2] Although these descriptions lacked modern detail, they projected the direction of the numerous investigations conducted since then. It is now confirmed that there is a generalized increase in permeability and there is a massive reorganization of the egg surface after fertilization (reviewed by Giudice[3] and Epel[4]).

Reports from this laboratory [5–7] and numerous other laboratories have confirmed that sea urchin eggs exhibit a massive increase in nucleoside uptake shortly after fertilization (reviewed by Nishioka *et al.*[8]). This increase provides an exam-

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ple, among many others, of a functional change at the egg surface following fertilization (reviewed by Nishioka[9]). The physiological role of increased nucleoside uptake is not readily obvious, since fertilized sea urchin eggs are able to develop in seawater devoid of nucleosides. Nevertheless, it has long been known that sea urchin embryos readily utilize exogenously supplied nucleosides in nucleic acid synthesis.[10] The increase may be a reanimation of a cellular process that was important during oogenesis when stores of metabolic precursors need to be accumulated. Additionally, Pardee *et al.*[11], among others,[12–14] have noted that increased nucleoside uptake is observed in virtually all proliferating animal cells, suggesting that it may represent a general response of mitogenically stimulated cells.

The rate of nucleoside uptake increases greater than 50-fold shortly after fertilization[5] and is concentration dependent,[15] temperature dependent,[16] Na^+-dependent,[5] and inhibitable by 2,4-dinitrophenol.[16,17] All of the deoxyribonucleosides and ribonucleosides normally present in DNA and RNA compete with the DNA-specific deoxyribonucleoside, thymidine, for transport sites. The free pyrimidine and purine bases, the deoxyribose and ribose sugars, the deoxyribonucleotides, and amino acids do not compete, showing that the specificity of this uptake lies at the nucleoside level.[8] Uptake of thymidine may be turned on in unfertilized eggs by treatment with low concentrations of ammonia which bypasses the Ca^{2+}-requiring egg cortical reaction and experimentally raises the intracellular pH. However, when compared with uptake in fertilized eggs, uptake in ammonia-treated eggs is stimulated later and to a lower rate. Both of these deficiencies may be reversed by a subsequent induction of the cortical reaction by fertilization or by experimental treatments with either butyric acid or the Ca^{2+}-ionophore A23187.[5,8] These results suggest that both the Ca^{2+}-requiring cortical reaction and increased intracellular pH are involved in the turn on of nucleoside uptake in fertilized eggs.

In heretofore unrelated studies, nitrobenzylthioinosine (NBMPR) and various similar compounds have been shown to act as potent and specific inhibitors of nucleoside uptake in human erythrocytes[18,19] and various other mammalian cell-types.[20–23] In HeLa cells, for example, the transport mediated component of adenosine uptake was eliminated in the presence of 5 μM NBMPR, revealing a non-saturable component of uptake which might represent simple diffusion.[20] HeLa cells possess sites to which NBMPR binds reversibly, but with high affinity (K_d about 0.1 nM), and NBMPR occupancy of these sites results in the inhibition of uptake of various nucleosides.[24]

The present study was undertaken to determine the effects of NBMPR on the uptake of thymidine in fertilized sea urchin eggs and to determine if NBMPR exerts any negative effects on the early development of embryos.

Materials and Methods

Experimental Sea Waters. Artificial sea water (ASW) was prepared according to the Woods Hole formula of Harvey:[25] 0.423 M NaCl; 0.009 M KCl; 0.00927 M $CaCl_2$; 0.02294 M $MgCl_2$; 0.0255 M $MgSO_4$; 0.00215 M $NaHCO_3$; pH 8.0. Na^+-free sea water ($0Na^+$-SW) was prepared according to the same formula, substituting choline chloride for NaCl and $KHCO_3$ for $NaHCO_3$. Ca^{2+}-free sea water ($0Ca^{2+}$-SW) was prepared according to the same formula, substituting NaCl for $CaCl_2$ to give a final NaCl concentration of 0.4323 M.

Procurement of Gametes. Sea urchins, *Strongylocentrotus purpuratus* were purchased from Pacific Biomarine Laboratories, Inc. (Venice, California) and maintained at 15°C in Instant Ocean aquaria containing Instant Ocean syn-

thetic sea water. Shedding of gametes was induced by intracoelomic injection of 0.55 M KCl. Semen was shed directly into Syracuse dishes and maintained ice cold and undiluted until use. Eggs were shed directly into ASW, dejellied by agitation, and allowed to settle through three changes of ASW at 15°C.

Fertilization and Parthenogenetic Treatment. Fertilization was achieved by adding 0.01 vol of stock sperm suspension (1 drop undiluted semen in 5 ml ASW) to 1% (v/v) egg suspensions in ASW. For parthenogenesis, a 5.0 mM stock solution of Ca^{2+}-ionophore A23187 (Calbiochem-Behring) was prepared in dimethyl sulfoxide and added to 1% egg suspensions to a final concentration of 20 μM. These procedures resulted in greater than 98% fertilization and parthenogenetic activation, as determined microscopically by the appearance of elevated fertilization coats.

Measurements of Uptake and Binding. Uptake of [^{3}H-methyl]-thymidine (ICN, 60 Ci/mmole) and binding of [^{3}H]-NBMPR (Moravek Biochemicals, 35-37 Ci/mmole) were measured in this study. Crystalline NBMPR (Aldrich) was used in inhibition experiments. For measurements of [^{3}H]-thymidine uptake, standard 1% (v/v) egg suspensions were prepared in the various experimental sea waters containing 1.0 μCi/ml [^{3}H]-thymidine and cultured at 15°C with swirling every 5 min. At timed intervals after fertilization or parthenogenetic activation, 5.0 ml samples were removed from the cultures, placed in 15 ml conical centrifuge tubes, and centrifuged at 300 × g for 1 min. After two 5 ml washings with the appropriate ice cold sea water, the egg pellets were suspended in 0.5 ml NCS tissue solubilizer (Amersham) : H_2O (9 : 1) and incubated at 50°C for 2 hr. The dissolved samples were transferred to scintillation vials with two 5 ml washings of scintillation fluid (5.0 g PPO, 0.1 g POPOP per liter toluene). Radioactivity in each sample was measured with a Beckman LS 7500 liquid scintillation counter. For measurements of [^{3}H]NBMPR binding, 1% egg suspensions containing 20 nM [^{3}H]-NBMPR were cultured at 15°C and stirred continuously at 60 rpm with motor-driven teflon paddles. At timed intervals after exposure to [^{3}H]-NBMPR, 2.5 ml samples were removed from the cultures and processed as described for uptake of [^{3}H]-thymidine, except that the sample pellets received only one wash with the appropriate ice-cold sea water.

Na^+-Free and Ca^{2+}-Free Experiments. In experiments involving Na^+-free or Ca^{2+}-free conditions, the eggs were washed three times with at least 50 vol $0Na^+$-SW or $0Ca^{2+}$-SW by centrifugation and aspiration of supernates. In experiments comparing binding under Na^+-free and Na^+-containing conditions, two cultures were set up under Na^+-free conditions and NaCl was added to one of the cultures to a final concentration of 50 mM. In experiments comparing binding or uptake under Ca^{2+}-free and Ca^{2+}-containing conditions, two cultures were set up under Ca^{2+}-free conditions and $CaCl_2$ was added to one of the cultures to a final concentration of 10 mM.

Results

Uptake of [^{3}H]-Thymidine

Figure 1 shows the cumulative uptake of [^{3}H]-thymidine in unfertilized and fertilized sea urchin eggs. Unfertilized eggs show a low, constant rate of uptake, while fertilized eggs exhibit a sharp increase 10 min after insemination which begins to plateau at 80–90 min. The plateau reflects the exhaustion of thymidine from the medium and demonstrates the extreme efficiency of this transport system. The difference in the amounts of uptake through the first 90 min between unfertilized and fertilized eggs represents a greater than 50-fold increase in the rate of thymidine uptake following fertilization. These results further confirm earlier reports from this laboratory.[5–8]

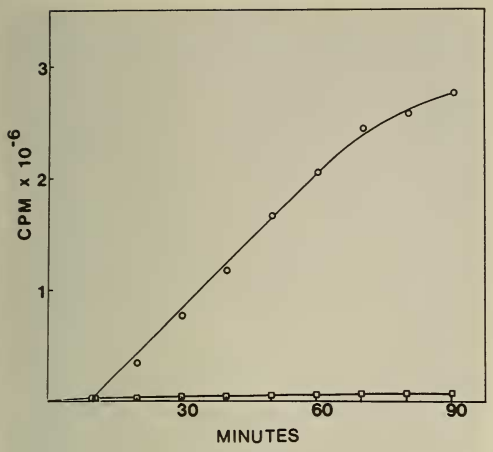

Fig. 1. Cumulative uptake of [^{3}H]-thymidine (1.0 μCi/ml, 60 Ci/mmole) in unfertilized [□] and fertilized [○] sea urchin eggs. Insemination is at time 0.

Effects of NBMPR on [^{3}H]-Thymidine Uptake

To determine the effects of NBMPR on thymidine uptake in fertilized eggs, the cumulative uptake of [^{3}H]-thymidine was measured at 60 min post-insemination in the presence of increasing concentrations of NBMPR. Figure 2 shows the reductions in uptake, and corresponding % inhibitions, as the concentration of NBMPR is raised from 0 to 400 μM. Uptake is reduced rapidly to 60% inhibition between 0 and 100 μM and continues to fall, although much less rapidly, to 80% inhibition between 100 and 400 μM.

Binding of [^{3}H]-NBMPR in 0Na$^+$-SW

Since a strict requirement for external Na^+ has been reported for thymidine transport[5] and since our results indicate that NBMPR is an effective inhibitor of this transport, the binding of [^{3}H]-NBMPR to fertilized eggs was compared under Na^+-containing and Na^+-free conditions. For this comparison, eggs were fertilized in ASW, washed three times with 0Na^+-SW, divided into two equal suspensions, and cultured at 15°C with constant stirring. At time 0, [^{3}H]-NBMPR (*q.s.* 20 nM) was added to one of the cultures and [^{3}H]-NBMPR (*q.s.* 20 nM) + NaCl (*q.s.* 50 mM) were added to the second culture. Figure 3 shows that there is no significant difference in NBMPR binding through a 45 min comparison. Apparently, binding of NBMPR to surface sites on fertilized sea urchin eggs is not Na^+-dependent.

Binding of [^{3}H]-NBMPR in 0Ca^{2+}-SW

When a similar comparison was made between Ca^{2+}-containing and Ca^{2+}-free

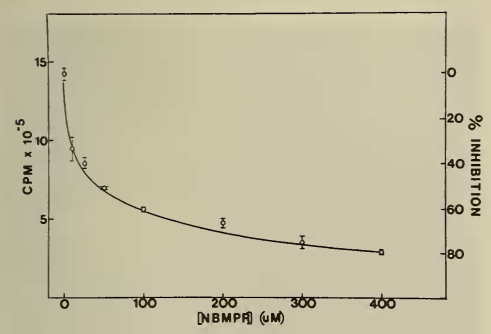

Fig. 2. Inhibition of [^{3}H]-thymidine uptake by nitrobenzylthioinosine (NBMPR). Cumulative uptake in fertilized sea urchin eggs exposed to increasing concentrations of NBMPR was measured at 60 min post-insemination. Error bars indicate standard deviations from the means for three samples measured at each NBMPR concentration.

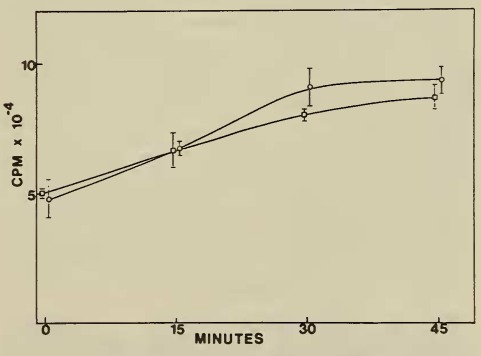

Fig. 3. [^{3}H]-NBMPR binding to fertilized sea urchin eggs suspended in 0Na^+-SW [□] and 0Na^+-SW containing 50 mM NaCl [○]. Error bars indicate standard deviations from the means for three samples measured at each timepoint.

conditions, a significant reduction in the amount of [^{3}H]-NBMPR binding was detected in 0Ca^{2+}-SW. For this comparison, unfertilized eggs were washed three times in 0Ca^{2+}-SW, divided into two equal suspensions and cultured at 15°C with constant stirring. At time 0, [^{3}H]-NBMPR (*q.s.* 20 nM) + ionophore A23187 (*q.s.* 20 μM) were added to one of the cultures and [^{3}H]-NBMPR (*q.s.* 20 nM) + ionophore A23187 (*q.s.* 20 μM) + $CaCl_2$ (*q.s.* 10 mM) were added to the second culture. Elevation of fertilization coats was greater than 98% in both cultures. Figure 4 shows a 50% reduction in [^{3}H]-NBMPR binding under Ca^{2+}-free conditions through a 45 min comparison. These results reveal for the first time the existence of a Ca^{2+}-dependent component of NBMPR binding.

Parthenogenetic activation by A23187 was chosen for these experiments because the sperm acrosome reaction and fertilization are inhibited in 0Ca^{2+}-SW. Additionally, fertilization coats fail to harden in 0Ca^{2+}-SW, making excessive clumping during transfer of fertilized eggs to 0Ca^{2+}-SW an operational difficulty.

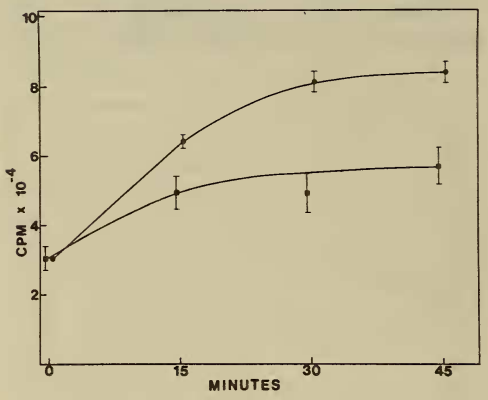

Fig. 4. [^{3}H]-NBMPR binding to ionophore A23187-activated sea urchin eggs suspended in 0Ca^{2+}-SW [■] and 0Ca^{2+}-SW containing 10 mM $CaCl_2$ [●]. Error bars indicate standard deviations from the means for three samples measured at each timepoint.

[^{3}H]-*Thymidine Uptake in 0Ca^{2+}-SW*

Since the binding of NBMPR was shown to be Ca^{2+}-dependent, the determination of whether thymidine uptake is also Ca^{2+}-dependent was made. Unfertilized eggs were washed three times in 0Ca^{2+}-SW and divided into four equal suspensions containing 1.0 μCi/ml [^{3}H]-thymidine and cultured at 15°C. At time 0, ionophore A23187 (*q.s.* 20 μM) was added to one of the cultures and ionophore A23187 (*q.s.* 20 μM) + $CaCl_2$ (*q.s.* 10 mM) were added to a second culture. The third and fourth cultures were used as unactivated controls with and without Ca^{2+}. Figure 5 shows that the stimulation of thymidine uptake in A23187-treated eggs under Ca^{2+}-containing conditions is similar to the stimulation observed in fertilized eggs suspended in ASW (compare with Fig. 1). Uptake in 0Ca^{2+}-SW, however, is nearly 20% reduced through the 45 min course of the experiment. These results reveal a Ca^{2+}-dependent compo-

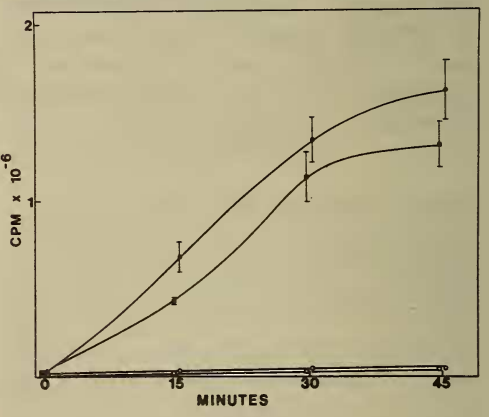

Fig. 5. Cumulative uptake of [^{3}H]-thymidine in sea urchin eggs: unfertilized eggs suspended in 0Ca^{2+}-SW [□]; unfertilized eggs suspended in 0Ca^{2+}-SW containing 10 mM $CaCl_2$ [○]; ionophore A23187-activated eggs suspended in 0Ca^{2+}-SW [■]; and ionophore A23187-activated eggs suspended in 0Ca^{2+}-SW containing 10 mM $CaCl_2$ [●]. A23187 activation is at time 0. Error bars indicate standard deviations from the means for three samples measured at each timepoint.